Bioorganic & Medicinal Chemistry Letters 18 (2008) 4581–4583

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

10-Hydroxy-7,8-dihydropyrazino[1′,2′:1,5]pyrrolo[2,3-*d*]pyridazine-1,9(2*H*,6*H*)-diones: Potent, orally bioavailable HIV-1 integrase strand-transfer inhibitors with activity against integrase mutants

Catherine M. Wiscount [a,*], Peter D. Williams [a], Lekhanh O. Tran [a], Mark W. Embrey [a], Thorsten E. Fisher [a], Vanessa Sherman [a], Carl F. Homnick [a], D. Donnette Staas [a], Terry A. Lyle [a], John S. Wai [a], Joseph P. Vacca [a], ZiQiang Wang [a], Peter J. Felock [b], Kara A. Stillmock [b], Marc V. Witmer [b], Michael D. Miller [b], Daria J. Hazuda [b], Alysha M. Day [b], Lori J. Gabryelski [c], Linda T. Ecto [c], William A. Schleif [c], Daniel J. DiStefano [c], Christopher J. Kochansky [d], M. Reza Anari [d]

[a] *Department of Medicinal Chemistry, WP14-3, Merck Research Laboratories, West Point, PA 19486, USA*
[b] *Department of Antiviral Research, Merck Research Laboratories, West Point, PA 19486, USA*
[c] *Department of Vaccine and Biologics Research, Merck Research Laboratories, West Point, PA 19486, USA*
[d] *Department of Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA*

ARTICLE INFO

Article history:
Received 14 May 2008
Accepted 10 July 2008
Available online 15 July 2008

Keywords:
HIV integrase
Antiviral

ABSTRACT

A series of 10-hydroxy-7,8-dihydropyrazino[1′,2′:1,5]pyrrolo[2,3-*d*]pyridazine-1,9(2*H*,6*H*)-diones was synthesized and tested for their inhibition of HIV-1 replication in cell culture. Structure–activity studies indicated that high antiviral potency against wild-type virus as well as viruses containing integrase mutations that confer resistance to three different structural classes of integrase inhibitors could be achieved by incorporation of small aliphatic groups at certain positions on the core template. An optimal compound from this study, **16**, inhibits integrase strand-transfer activity with an IC_{50} value of $\leqslant$10 nM, inhibits HIV-1 replication in cell culture with an IC_{95} value of 35 nM in the presence of 50% normal human serum, and displays modest pharmacokinetic properties in rats (iv $t_{1/2}$ = 5.3 h, F = 17%).

The causative agent of acquired immune deficiency syndrome (AIDS) is the human immunodeficiency virus type 1 (HIV-1). Resistance to marketed anti-HIV drugs is increasing at an alarming rate and thus there is a need to improve existing agents and develop new agents which work by different mechanisms. The viral enzyme, integrase, is used by the virus to insert double-stranded proviral DNA into host chromosomal DNA. Several integrase inhibitors which work by inhibiting the strand-transfer step have progressed to the stage of clinical testing in patients infected with HIV-1, and one of these, Raltegravir™, has recently received FDA approval.[1] We have been working toward second-generation integrase strand-transfer inhibitors with the goal of identifying inhibitors with activity against viruses that contain integrase mutations. A recent report from our laboratories[2] described tricyclic hydroxypyrrole **1** as a novel mimetic of the metal binding pharmacophore[3] of diketoacid integrase inhibitors (Fig. 1). Scaffold **1** offers unique opportunities to maintain activity against integrase mutants because it may take advantage of multiple binding modes due to its pseudo-C_2 symmetry.[2] However, in follow-up investigations we found that alkali metal salts of **1**, which were desired for oral dosing in pharmacokinetic experiments, were susceptible to air oxidation. We reasoned that introduction of an electron withdrawing group into the ring system, such as contained in pyridazinone analog **2**, would stabilize the electron-rich hydroxypyrrole moiety and attenuate oxidation. Indeed, alkali metal salts of scaffold **2** were found to possess markedly improved chemical stability. Herein

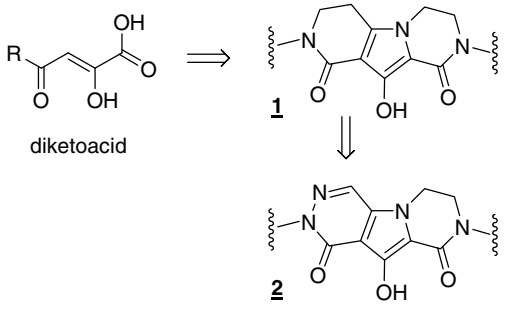
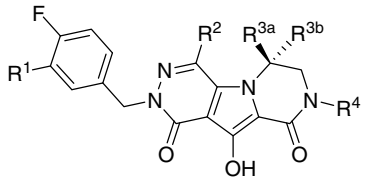


Figure 1.

* Corresponding author. Tel.: +1 215 652 7812; fax: +1 215 652 3971.
E-mail address: cathy_wiscount@merck.com (C.M. Wiscount).

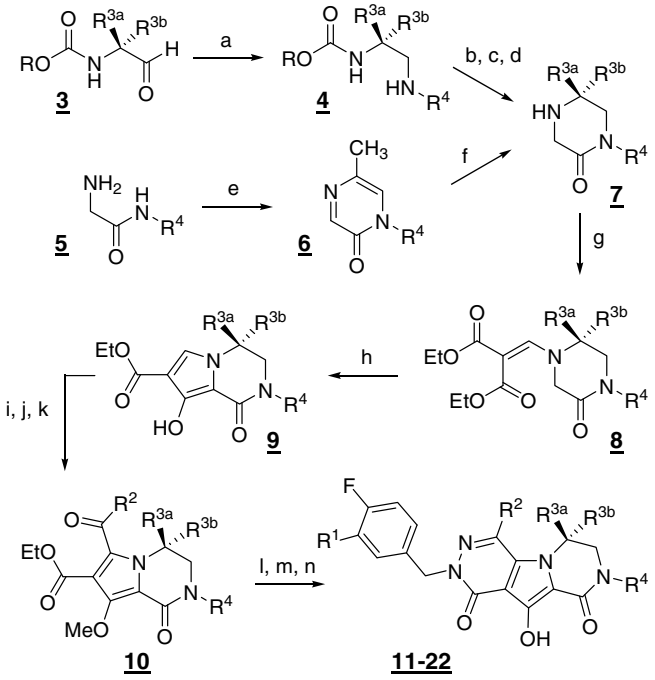
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.07.037

Table 1
Antiviral potency of pyrazino-pyrrolopyridazines in cell culture assays



Compound	R^1	R^2	R^{3a}	R^{3b}	S:R[a]	R^4	Viral replication assays					
							Multi-cycle[b]		Single cycle[c]			
							IC_{95} (nM)		IC_{50} (nM)	Fold shift vs WT		
							10% FBS	50% NHS	WT	F121Y	T66I S153Y	N155S
11	H	H	H	H	—	Me	120	380	72	11	7	53
12	H	Me	H	H	—	Me	43	130	21	10	8	48
13	H	Me	Me	H	99:1	Me	10	31	10	4	2	20
14	H	Me	H	Me	1:99	Me	250	1000	66	9	8	91
15	H	Me	Me	H	97:3	Et	17	40	14	1	1	6
16	H	Me	Me	H	95:5	*i*-Pr	16	35	10	1	1	2
17	H	H	Me	H	99:1	Et	24	46	16	5	1	27
18	H	H	H	Me	1:99	Et	250	500	52	8	9	10
19	H	H	Me	Me	—	Et	10	30	19	10	3	27
20	H	H	Me	H	95:5	*i*-Pr	13	26	19	2	1	6
21	Cl	H	Me	H	97:3	Et	31	160	19	2	1	7
22	Cl	H	Me	H	95:5	*i*-Pr	30	220	28	1	1	3

[a] Ratio of *S* and *R* enantiomers as analyzed on a Chiracel OD column.
[b] Antiviral activites in MT-4 cells cultured in the presence of 10% fetal bovine serum (FBS) or 50% normal human serum (NHS) and infected with an HIV-1 H9/IIB virus. All values reported are means of three or more independent determinations.
[c] Antiviral activities in P4/5 cells cultured in the presence of 10% FBS and infected with an HIV-1 IIIB virus. Fold-shift values are defined as IC_{50} (mutant)/IC_{50} (wild type).



Scheme 1. Reagents and conditions: (a) R^4NH_2, $NaBH(OAc)_3$, AcOH, THF or CH_2Cl_2 (81–89%); (b) $BrCH_2CO_2H$, DCC, CH_2Cl_2 or $BrCH_2COBr$, Et_3N, THF (84–91%); (c) NaH, THF or DMF (61–85%); (d) HCl, EtOAc (R = *t*-Bu) or H_2, Pd–C, MeOH (R = CH_2Ph) (91–98%); (e) pyruvaldehyde, NaOH, H_2O, 100 °C (84%); (f) H_2, PtO_2, EtOAc (92%); (g) diethyl ethoxymethylidene-malonate, $EtN(i\text{-}Pr)_2$, toluene, 80 °C (85–89%); (h) $LiN(TMS)_2$, THF, 50 °C (50–65%); (i) MeI, K_2CO_3, DMF (88–93%); (j) Br_2, $NaHCO_3$, CH_2Cl_2 (82–90%); (k) R^2 = H:$Bu_3SnCH{=}CH_2$, $(t\text{-}Bu_3P)_2Pd$, toluene, microwave 100 °C, then OsO_4, $NaIO_4$, THF:H_2O (71–79%); (k) R^2 = CH_3:*n*-BuOCH=CH_2, $(t\text{-}Bu_3P)_2Pd$, toluene, microwave 100 °C, then AcOH, THF:H_2O; or $Bu_3SnC(OMe){=}CH_2$, $PdCl_2(PPh_3)_2$, DMF, microwave 100 °C (78–87%); (l) $N_2H_2{\cdot}H_2O$, microwave 100 °C (78–95%); (m) 4-F- or 4-F, 3-Cl-benzyl bromide, Cs_2CO_3, DMF (82–91%); (n) BBr_3, CH_2Cl_2 (80–95%).

we present SAR findings with scaffold **2** that demonstrate a pathway to potent, orally bioavailable integrase inhibitors with activity against a selected panel of integrase mutants.

Chemical synthesis (Scheme 1)*:* Key early intermediates for syntheses of the compounds in Table 1 were piperazinones **7**. Conversion to enamides **8** and base-induced ring closure gave pyrrolopiperazines **9**. The hydroxyl group was methylated, the pyrrole brominated, and the bromide underwent Stille- or Heck-type couplings to give acyl pyrroles **10**. Ring closure to the tricyclic core was achieved by treating **10** with hydrazine hydrate. N-Benzylation followed by demethylation gave inhibitors **11**–**22**. Enantiomer pairs **13**/**14** and **17**/**18** were obtained by separation of the racemates on a Chiracel OD column. The racemic piperazinones **7** utilized in these syntheses were obtained by hydrogenation of pyrazinones **6**.[4] The absolute stereochemistry was established by asymmetric syntheses starting with *S*-aldehyde **3**[5] and using a four-step sequence[6] to obtain enantiomerically enriched piperazinones **7**. Enantiomeric purity was assessed using a Chiracel OD column at the final product stage and was found to be ~95:5 *S*:*R*, see Table 1 for specific ratios. An analogous piperazinone synthesis starting with *N*-Cbz-dimethylglycinal provided the achiral inhibitor **19**. Syntheses of **11** and **12** began with commercially available 1-methyl-2-piperazinone.

Biological methods: The compounds in Table 1 were tested for antiviral efficacy in two cell-based assays. No evidence of compound-related cytotoxicity was observed in either assay. Multi-cycle viral replication assays[7] were performed in MT-4 human T lymphoid cells cultured in media containing 10% fetal bovine serum (FBS) or 50% normal human serum (NHS) and infected with HIV-1 H9/IIIB virus. Inhibition of viral growth was assessed by measuring P24 levels with an ELISA. Single-cycle viral replication assays[8] were performed in P4/R5 cells (HeLa cells with a stably integrated LTR-LacZ reporter gene) cultured in a medium containing 10% FBS and infected with HIV-1 IIIB virus. Viral growth was measured using a β-galactosidase readout. In single-cycle infectiv-

Table 2
Pharmacokinetic parameters of selected compounds in male Sprague–Dawley rats[a]

Compound	CL (mL/min/kg)	V_d (L/kg)	$t_{1/2}$ (h)	%F
15	17	2.6	6.2	7
16	3.0	0.3	5.3	17
17	0.50	0.2	6.0	31
19	0.68	0.3	5.2	13
20	0.17	0.2	12	21
21	0.27	0.3	9.5	13
22	0.17	0.2	13	14

[a] Compounds were dosed orally as sodium salts at a dose of 10 mg/kg in 0.5% methocel suspension and intravenously as parent phenols at a dose of 2 mg/kg in 1:2 DMSO:H_2O solution. Clearance, volume of distribution, and half-life values were calculated from the intravenous experiments.

ity experiments, wild-type virus as well as viruses containing integrase mutations that confer resistance to diketoacid and naphthyridine strand-transfer inhibitors[8] were used to assess potency of test compounds. Selected compounds were tested for pharmacokinetic behavior in male Sprague–Dawley rats. Compounds were dosed orally as sodium salts in 0.5% methocel suspension at 10 mg/kg and intravenously as parent phenols in 1:2 DMSO:H_2O solution at 2 mg/kg, using three animals per dose group. Plasma drug levels were measured at 10 time points between 0.2 and 24 h in the intravenous experiments and at eight time points between 0.2 and 24 h in the oral experiments.

Results and discussion: The parent compound in this series, **11**, demonstrated that this scaffold is capable of producing good antiviral activity in cell culture with minimal shift in potency in the presence of 50% normal human serum (NHS). However, **11** suffers a 10- to 50-fold loss in potency against three integrase mutants, F121Y, N155S, and T66I/S153Y. Addition of a methyl group at R^2 on the pyridazinone ring (**12**) improved antiviral potency by 3-fold but did not improve potency against the mutants. Addition of another methyl group at R^3 on the piperazinone ring had a significant effect on potency: compared to **12** in the multi-cycle assay, the *S* isomer (**13**) gained 4-fold in potency whereas the *R* isomer (**14**) lost 5-fold in potency. A similarly large difference in potency favoring the *S*-isomer was observed with enantiomers **17** and **18**, and we therefore focused our attention exclusively on analogs in the *S*-series for additional structure-activity studies. Two analogs of **13** with larger piperazinone N-substituents at R^4, **15** and **16**, maintained antiviral potency and showed significantly improved potency against the three integrase mutants. In pharmacokinetic experiments (see Table 2), **15** and **16** exhibited modest oral bioavailability with moderate to low clearance, and half-lives in the range of 5–6 h. Compounds **15** and **16** thus demonstrated that this series was capable of delivering orally bioavailable analogs with high antiviral potency and excellent activity against integrase mutants. In the related series with R^2 = H, the antiviral potency of the achiral *gem*-dimethyl analog **19** compares more closely to that of the *S*-monomethyl analog **17** than the *R*-monomethyl analog **18**. The *gem*-dimethyl analog **19** did not provide any advantage for potency against the mutants. In comparing the potencies of the R^4 = ethyl and isopropyl analogs in the two series with R^2 = H or methyl (**17** and **20** vs **15** and **16**), it is seen that that there is a slight advantage for the series with R^2 = methyl in terms of antiviral potency and activity against mutants. However, **17** and **20** in the R^2 = H series exhibited lower clearance in pharmacokinetic experiments in rats. Additionally, **20** exhibited a long plasma half-life of 12 h after intravenous administration. The chloro analogs, **21** and **22**, demonstrated an upper limit for lipophilicity: in the multi-cycle viral replication assay these two compounds exhibited a 5- to 6-fold shift when comparing potencies in the 10% FBS and 50% NHS experiments, whereas the *des*-chloro analogs in Table 1 exhibited a shift of only 2- to 3-fold. The greater shift for **21** and **22** suggests that they are more highly bound to serum proteins than the *des*-chloro analogs and their antiviral potencies in the presence of human serum are less optimal (IC_{95} >100 nM). Overall, the two *N*-isopropyl analogs **16** and **20** exhibited a favorable combination of properties from the standpoint of antiviral potency in the presence of 50% NHS, potency against the three mutants, and pharmacokinetic properties in rats. Both compounds were additionally characterized in an integrase-catalyzed strand-transfer assay[9] and exhibited potent inhibition with IC_{50} values $\leqslant$10 nM (lower limit of the assay). In a single-cycle replication assay using virus that incorporates a Q148K mutation in integrase[10], a mutation which confers ~50-fold resistance to Raltegravir™, **16** and **20** showed differential activity with shifts versus wild-type virus of 3- and 45-fold, respectively. In pharmacokinetic experiments in male beagle dogs, poor oral bioavailability (<5%), moderate clearance (~10 mL/min/kg), and short intravenous half-lives (~1 h) were observed for both **16** and **20**.

Summary and conclusion: Modification of a previously reported pyrazino-pyrrolopyrazine integrase inhibitor by changing one of the pyrazinone rings to a pyridazinone improved chemical properties such that stable alkali metal salts could be prepared and used for oral dosing in pharmacokinetic experiments. Addition of small aliphatic groups to the pyrazinopyrrolopyridazine core provided a means to obtain potent antiviral activity in cell culture against wild-type virus as well as viruses which contain mutations in integrase that confer resistance to integrase inhibitors from three different structural classes. From these studies, **16** was found to possess a number of favorable attributes: antiviral IC_{95} = 35 nM in the presence of 50% NHS against HIV-1 in cell culture; 1- to 3-fold shift in potency against viruses that contain resistance-conferring mutations to diketoacid, naphthyridine, and pyrimidine classes of integrase inhibitors; and modest oral bioavailability and plasma half-life in rats (F = 17%, intravenous $t_{1/2}$ = 5.3 h). Compound **16** thus represents a promising lead for further optimization studies toward second-generation integrase strand-transfer inhibitors.

References

1. Anthony, N. J. *Curr. Top. Med. Chem.* **2004**, *4*, 979; Egbertson, M. S. *Curr. Top. Med. Chem.* **2007**, *7*, 1251.
2. Fisher, T. E.; Kim, B.; Staas, D. D.; Lyle, T. A.; Young, S. D.; Vacca, J. P.; Zrada, M. M.; Hazuda, D. J.; Felock, P. J.; Schleif, W. A.; Gabryelski, L. J.; Anari, M. R.; Kochansky, C. J.; Wai, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6511.
3. Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S. D.; Vacca, J. P.; Hazuda, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661.
4. Armarego, W. L. F.; Waring, P.; Williams, J. W. *J. Chem. Soc. Chem. Commun.* **1980**, *8*, 334.
5. Fehrentz, J. A.; Castro, B. *Synthesis* **1983**, *8*, 676.
6. Williams, T. M.; Bergman, J. M.; Brashear, K.; Breslin, M. J.; Dinsmore, C. J.; Hutchinson, J. H.; MacTough, S. C.; Stump, C. A.; Wei, D. D.; Zartman, C. B.; Bogusky, M. J.; Culberson, J. C.; Buser-Doepner, C.; Davide, J.; Greenberg, I. B.; Hamilton, K. A.; Koblan, K. S.; Kohl, N. E.; Liu, D.; Lobell, R. B.; Mosser, S. D.; O'Neill, T. J.; Rands, E.; Schaber, M. D.; Wilson, F.; Senderak, E.; Motzel, S. L.; Gibb, J. B.; Graham, S. L.; Heimbrook, D. C.; Hartman, G. D.; Oliff, A. I.; Huff, J. R. *J. Med. Chem.* **1999**, *42*, 3779.
7. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabac, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lim, J.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. E.; Huff, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.
8. Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I.-W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233.
9. Hazuda, D. J.; Felock, P.; Hastings, J. C.; Pramanik, B.; Wolfe, A. *J. Virol.* **1997**, *71*, 7005.
10. Ke, Y; Danovich, R. M.; personal communication. See also Cooper, D., Gatell, J.; Rockstroh, J.; Katlama, C.; Yeni, P.; Lazzarin, A.; Chen, J.; Isaacs, R.; Teppler, H.; Nguyen, B.; *14th Conference on Retroviruses and Opportunistic Infections*, February 25–28, 2007.